5. [Amended] The monobody of claim 1, wherein the monobody loop region [sequences vary] sequence varies from the wild-type Fn3 loop region [sequences] sequence by the deletion or replacement of [at least] 2 to 25 amino acids.

6. [Amended] The monobody of claim 1, wherein the monobody loop region [sequences vary] sequence varies from the wild-type Fn3 loop region [sequences] sequence by the insertion of from 3 to 25 amino acids.

REMARKS

Applicant has carefully reviewed and considered the Office Action mailed on August 18, 2000, and the references cited therewith.

Claims 1-6 are amended, and claims 7-39 are canceled; as a result, claims 1-6 are now pending in this application. No new subject matter has been added to the claims. The amendments to the claims are fully supported by the specification as originally filed. The amendments are made to clarify the claims, and are not intended to limit the scope of equivalents to which any claim element may be entitled. In accordance with patent parlance as interpreted by the Federal Circuit, applicants intend that the indefinite article "a" or "an" carries the meaning of "one or more." *KCJ Corporation v. Kinetic Concepts, Inc.*, 223 F.3d 1351, 1356; 55 U.S.P.Q.2d 1835 (Fed. Cir. 2000).

Applicant respectfully requests reconsideration of the above-identified patent application as amended and in view of the following remarks.

Information Disclosure Statement

In response to the Examiner's objection concerning the lack of information provided for the Koide et al. reference, attached is a substitute for the Form 1449 originally submitted February 15, 1999, along with a copy of the cited reference and a more complete citation. It is respectfully requested that the Examiner initial and return a copy of this Form 1449 with his next action.

Specification

The specification has been amended in accordance with the Examiner's requests. In particular, the first line of the specification is updated to indicate the priority information. Also, all trademarked terms are capitalized. Further, the Brief Description of the Drawings has been amended.

In the Drawings

The objections to the drawings are noted. Corrected drawings will be submitted upon receipt of a Notice of Allowance.

§112, Second Paragraph, Rejection of Claims

Claims 1-6 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner states that claims 1-6 are indefinite because claim 1 recites "plurality of Fn3 . . . linked to a plurality of loop region sequences". Claim 1 has been amended rendering this rejection moot.

The Examiner states that claims 1-5 are indefinite for reciting "deletion, insertion, or replacement of at least two amino acids" in claim 1 and "deletion or replacement of at least 2 amino acids" in claim 5. Claims 1 and 5 have been amended to recite "two to 25 amino acids" to clarify the claim.

The Examiner states that there is insufficient basis for the phrase "one or more of the loop regions." This phrase has been deleted.

The Examiner states that claims 2 and 3 are indefinite for reciting the word "capable." This word has been deleted from the claims.

The Applicant requests that the rejections under 35 U.S.C. § 112, second paragraph be withdrawn.

§112, First Paragraph, Rejection of Claims

Claims 1-6 were rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for a fibronectin type III polypeptide monobody comprising the tenth fibronectin unit of human fibronectin wherein the monobody loop regions of residues 22-30 in the BC loop and residues 76-87 in the FG loop vary by substitutions, insertions, or replacement from the wild-type FN3 by 3 to 25 amino acids, wherein the replaced loop region amino acid residues bind to a specific binding partner and do not affect the proper folding of the monobody, does not reasonably provide enablement for a fibronectin type III polypeptide monobody comprising a structure of less than the FN3 domain, or wherein any amino acids in any loop region other than the BC or FG loops have any amino acid deletions, insertions, or replacements, or wherein the beta-strand domains have 50% sequence homology to the wild-type beta-strand domain or wherein any loop region is capable of catalyzing a chemical reaction such that the ration of kcat/kuncat is greater than 10.

It is well established that two proteins with the amono acid identity greater than 30 % have almost always have the same global fold (Marti-Renom, M. A., Stuart, A. C., Fiser, A., Sanchez, R., Melo, F. & Sali, A. (2000) Comparative protein structure modeling of genes and genomes. *Annu Rev Biophys Biomol Struct 29*, 291-325. *See*, page 297 and, Figure 3 on page 307). Thus the examiner's statement, "Protein chemistry is probably one of the most unpredictable areas of biotechnology and as such it is unpredictable what the outcome of replacements will be or if a protein with 50% homology will function as claimed or be structurally related" is not correct. In addition, it is well known that conservative (homologous) mutations do not alter the conformational stability of a protein to a great degree.

The Examiner presented the following examples:

lysine to glutamic acid (Burgess et al; positively charged to negatively charged),
aspartic acid to serine or glutamic acid (Lazar et al; short, negatively charged to neutral, or long, negatively charged),
histidine to aspartic acid (Schwartz et al; large, positively charged to small, negatively charged), and
removal of the amino terminal histidine (Lin et al; removal of a large, positively charged residue, and movement of the amino terminal charge by one residue),

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All of these examples, however, involve changes in the sign of charge on a residue, an increase in the side chain size, and/or a removal of a large side chain. These are NOT generally considered "inconsequential chemical modifications". Moreover, these examples only teach that one could modulate a biological activity of a protein by a single mutation presumably at positions critical to the activity, but they fail to show that one cannot find mutations that retain the activity. Shortle et al. demonstrated that a large number of mutations marginally alter the conformational stability of Staphylococcal nuclease (Shortle, D., Stites, W. E. & Meeker, A. K. (1990) Contribution of the large hydrophobic amino acids to the stability of Staphylococcal nuclease. *Biochemistry 29*, 8033-8041; Green, S. M., Meeker, A. K. & Shortle, D. (1992) Contributions of the polar, uncharged amino acids to the stability of staphylococcal nuclease: evidence for mutational effects on the free energy of the denatured state. *Biochemistry 31*, 5717-28.). Matthews also demonstrated similar results on another protein T4 lysozyme (Matthews, B. W. (1993) Structural and genetic analysis of protein stability. *Ann. Rev. Biochem. 62*, 139-160).

Very recently, Cota et al. (Cota, E., Hamill, S. J., Fowler, S. B. & Clarke, J. (2000) Two proteins with the same structure respond very differently to mutation: the role of plasticity in protein stability. *J Mol Biol 302*, 713-25) showed that the tenth FN3 of human fibronectin ("FNfn10"), the identical protein that was used by the present inventor, can tolerate many mutations in the β-strand regions. Also FNfn10 is highly stable, suggesting that FNfn10 can even tolerate a few highly destabilizing mutations.

The FN3 domain is one of the most extensively studied protein domains. A large body of the literature exists that teaches methods to identify potential FN3 domains (Bork, P. & Doolittle, R. F. (1992) Proposed acquisition of an animal protein domain by bacteria. *Proc. Natl. Acad. Sci. USA 89*, 8990-8994; Bork, P. & Doolittle, R. F. (1993) Fibronectin type III modules in the receptor phosphatase CD45 and tapeworm antigens. *Protein Sci 2*, 1185-7; Bork, P., Hom, L. & Sander, C. (1994) The immunoglobulin fold. Structural classification, sequence patterns and common core. *J. Mol. Biol. 242*, 309-320; Campbell, I. D. & Spitzfaden, C. (1994) Building proteins with fibronectin type III modules. *Structure 2*, 233-337; Doolittle, R. F. (1995) The multiplicity of domains in proteins. *Annu Rev Biochem 64*, 287-314.).

Further, the FSSP Structural Alignment program,
`http://jura.ebi.ac.uk:8765/holm/qz?filename=/data/research/fssp//1fnf.fssp`

as of November 8th, 2000, identifies a total of 163 protein structures as a homolog of the FN3 structure. Campbell and Spetzfaden (Campbell & Spitzfaden, 1994) noted on page 333 that "[I]t appears that the Fn3 module is a stable and convenient fold, used in different ways to provide both an adaptable functional surface and a spacer." Taken together, it is reasonable for a person skilled in the art to expect that many mutations in the β-strand domain of FN3 have a minor effect on the activity and thus one can engineer mutant FN3 that retain the binding function by introducing mutations in the β-strand domain.

In addition to the examples presented in the original filing, the inventor has performed additional experiments that further support the pending claims. *See* Declaration under 37 C.F.R. § 1.132 of Dr. Koide, ¶2. The inventor prepared mutant FNfn10 proteins that contain glycine insertions in the BC, DE or FG loops. *Id.* Glycines were chosen to be inserted because glycine insertions are generally highly destabilizing. *Id.* Glycines have a high degree of conformational freedom (large entropy) in the unrestrained state. *Id.* It is thus energetically unfavorable to restraine glycines by folding of a protein, because of a high entropic penalty. *Id.*

Mutant proteins were prepared using the Kunkel mutagenesis and the conformational stability was determined using guanidine hydrochloride-induced denaturation, as described in (Koide, A., Bailey, C. W., Huang, X. & Koide, S. (1998) The fibronectin type III domain as a scaffold for novel binding proteins. *J. Mol. Biol. 284*, 1141-1151). Koide Declaration at ¶3. The Figure attached to the Koide Declaration shows effects of these mutations on the conformational stability of FNfn10. Specifically, the Figure depicts denaturation curves of FNfn10 and its variants containing additional glycine residues in one or more loops. *Id.* Denaturation reactions were monitored using tryptophan fluorescence and analyzed according to the two-state model, as described in Koide et al (1998). *Id.* Experiments were performed in 10 mM sodium citrate buffer pH 6.0 containing 100 mM sodium chloride at 30 °C. *Id.*

The following Table A summarizes the free energy of unfolding in the absence of guanidine hydrochloride. Koide Declaration at ¶4.

Table A: Effects of glycine insertions on the conformational stability of FNfn10

Protein	ΔG (kcal/mol)
Wild Type	7.70±0.09
Four glycines in the FG loop	6.91±0.15
Eight glycines in the FG loop	6.62±0.11
Four glycines in the DE loop	5.66±0.21
Four glycines in the BC loop and eight glycines in the FG loop	6.09±0.17

These results show that these insertions are in fact destabilizing, but these mutant FNfn10 proteins are still highly stable. *Id.* Thus, these results demonstrate that it is feasible to make mutant proteins in which the BC, DE and/or FG loops contain deletions, insertions and replacements. *Id.*

Recently Cota et al. (Cota, E., Hamill, S. J., Fowler, S. B. & Clarke, J. (2000) Two proteins with the same structure respond very differently to mutation: the role of plasticity in protein stability. *J Mol Biol 302*, 713-25) demonstrated that residues in the EF loop (residues 52, 64 and 66) can be replaced with alanine without detrimental effects on the conformational stability.

Taken together, both the inventor's results and those of Cota et al. demonstrate that FNfn10 can tolerate mutations in at least four out of the six loops. These data show that any loop in FNfn10 can be mutated without detrimental effects on the stability, supporting the pending claims.

Furthermore, an insertion of eight glycine residues in the FG loop (resulting in a 21-residue loop) did not cause a large decrease in stability. These results strongly suggest that one can insert even larger number of amino acid residues in FNfn10 loops. Thus, the inventor's results support claims for making monobodies in which one or more of the loops are extended by many residues.

Applicant requests that the rejections under 35 U.S.C. § 112 be withdrawn.

§102 Rejection of Claims

Claims 1-6 were rejected under 35 U.S.C. § 102(a) or (b) as being anticipated by Koide et al. (IDS #5). This rejection is traversed.

The Examiner is respectfully requested to consider the Rule 132 Declaration enclosed herewith, which is submitted to document the date on which the Koide et al. abstract was publicly available. In the Declaration, Anne Koch, an employee of Applicant's Representatives, states that she was informed by administrative personnel employed by the publisher of *The FASEB Journal* that the date on which Koide et al. abstract was mailed from the printer was August 7, 1997. The print version was available to the public on August 24, 1997. As the filing date of the priority document, U.S. provisional application 60/049,410, is June 12, 1997, the Koide et al. abstract is not prior art to the present application. Accordingly, Applicant respectfully requests withdrawal of the 35 U.S.C. § 102(a) rejection of the claims.

§103 Rejection of Claims

Claims 1-6 were rejected under 35 U.S.C. 103(a) as being unpatentable over Main et al., *Cell* 71:671-678 (1992) and further in view of Lee et al., *Protein Engineering*, 6:745-754 (1993).

Applicant respectfully submits that the Examiner has not established the *prima facie* obviousness of the present claims. To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the cited references themselves or in the knowledge generally available to an art worker, to modify the reference or to combine reference teachings so as to arrive at the claimed invention. Second, the art must provide a reasonable expectation of success. Finally, the prior art reference must teach or suggest all the claim limitations. *In re Ochiai*, 37 U.S.P.Q.2d 1127 (Fed. Cir. 1997) (When evaluating the scope of a claim, every limitation in the claim must be considered.).

The claims of the present application recite a monobody having a domain sequence linked to a loop region sequence, where the loop region is modified. The domain sequence, or "scaffolding," has at least a 50% total amino acid sequence homology to the corresponding amino acid sequence of wild-type Fn3's β-strand domain sequence.

Main et al. describe the three-dimensional structural of the tenth type III module of natural, wild-type human fibronectin. The authors state that the type III module contains a

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functional Arg-Gly-Asp (RGD) sequence known to be involved in cell adhesion. This, however, is the only adhesion-active sequence discussed in the paper. Main et al. do not teach or suggest that one could engineer binding molecules by altering loop sequences of the Fn3 scaffold. They do not even mention the possibility of mutating the Fn3 molecule. Thus, the claims are not obvious over Main et al.

Like Main et al., Lee et al., also focuses on the binding ability of RGD sequences to receptors. They teach installing the RGD sequence into "presentation scaffolds," which are small proteins of known 3-D structure chosen to present guest sequences in constrained orientations. (*See* abstract.) In particular, Lee et al. describe the design, construction and binding analysis of a series of mutants in which the RGD sequence has been inserted into two proteins of known structure: the immunoglobulin light chain variable (V_L) domain REI and interleukin-1β (IL-1β). Lee et al. at page 745, second column. Thus, Lee et al. focus on the problem of attempting to present the RGD binding sequence so that it will bind to its ligand, rather than on the problem of attempting to identify a scaffold sequence that could effectively present a variety of different binding sequences.

Lee et al., in fact, teach away from the present solution. Lee et al. at page 745, second column state the following:

> The concept of using a protein of known 3-D structure to present bioactive peptide sequences in constrained, structurally definable conformations has been introduced as one means to address this difficulty. Those hybrid molecules which exhibit good binding to the receptor can in principle be probed for their structures and structural dynamics to provide clues to conformational requirements for binding. Although this approach is conceptually attractive, it is not clear whether a presentation molecule can display an installed peptide sequence which allows receptor binding while at the same time providing sufficient structural interactions to constrain conformation. In addition, barriers to correct folding imposed by the inserted sequence may foil such experiments.

Thus, Lee et al. identify the problem, but not the solution, that the present inventors solve: a presentation molecule can display an installed peptide sequence that allows receptor binding, while at the same time providing sufficient structural interactions to constrain conformation.

Thus, even when combined, Main et al. and Lee et al. do not teach or suggest the present invention. Main et al. disclose the natural, wild-type tenth type III module of human fibronectin, and Lee et al. teach a constant binding region (RGD) that can be attached to various protein scaffolds, whereas the present invention teaches a constant protein scaffold that can be attached to various binding regions. Therefore, the present invention is novel and nonobvious. Accordingly, Applicant respectfully requests withdrawal of the 35 U.S.C. § 103(a) rejection of the claims.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612-373-6961) to facilitate prosecution of this application.

If necessary please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

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CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on this 20th day of November, 2000. (Monday)

Candis B. Buending
Name

Signature